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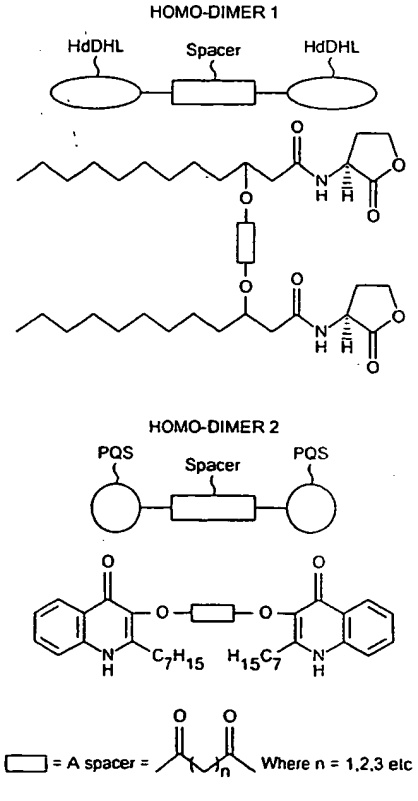
(54) Title: USE OF N-ACYL HOMOSERINE LACTONES FOR THE TREATMENT OF INSULITIS



(57) Abstract: An immune response modulatory compound is described. The compound has been shown to inhibit lymphocyte proliferation and to down-regulate TNF- secretion by monocytes and/or macrophages with the consequent activation of Th 1 lymphocytes in humans or other animals.

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USE OF N-ACYL HOMOSERINE LACTONES FOR THE TREATMENT OF INSULITIS

The invention relates to *N*-acyl homoserine lactones which have immunosuppressant properties and to pharmaceutical compositions containing them.

Immunosuppressant compounds induce an inhibition of the immune response system. Compounds which are known to exhibit immunosuppressant activity include the fungal metabolite Cyclosporin A and the macrolide antibiotic (a metabolite from *Streptomyces tsukabaensis*) termed FK506. Both of these agents have been used clinically and experimentally to suppress the immune system in transplantation and in the treatment of a number of diseases.

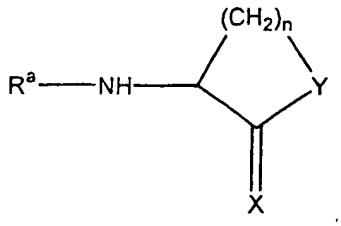
Autoimmune diseases are disorders where the host discrimination of "self" versus "non-self" breaks down and the individual's immune system (both acquired and innate components) attacks self tissues. These diseases range from common entities such as rheumatoid arthritis, thyroid autoimmune disease and type 1 diabetes mellitus to less common entities such as multiple sclerosis and to rarer disorders such as myasthenia gravis. Advances in basic biomedical science and, in particular, in immunology have indicated that the main and fundamental lesion responsible for the induction and persistence of most autoimmune diseases resides within auto-reactive proliferating T lymphocytes. In fact, the majority of autoimmune diseases are linked to a loss of T cell homeostasis. The healthy immune system is held in balanced equilibrium, apparently by the contra-suppressive production of cytokines by T helper 1 (Th1) and T helper 2 (Th2) lymphocyte subsets. In autoimmunity, Th1 cytokines predominate; in allergy, Th2 cytokines take their place. A cytokine intimately associated with the development of Th1 biased responses and, consequently, autoimmune disease is TNF-α.

Both Cyclosporin A and FK506 have been used clinically in the treatment of autoimmune diseases with encouraging results.

The currently available immunosuppressant drugs have the disadvantage of a narrow therapeutic index, i.e., toxicity versus clinical benefit. The compounds are known to be nephrotoxic, neurotoxic and potentially diabetogenic and this

has limited their use in the fields mentioned above. Problems also exist with the administration of these compounds, their bioavailability and the monitoring of their levels both clinically and in the laboratory.

We disclosed, in WO-A-95/01175, a class of compounds which exhibit antiallergic activity and inhibit the release of histamine, having the generic formula



where: n is 2 or 3; Y is O, S or NH; X is O, S or NH; and R^a is C_1-C_{18} alkyl or acyl which may be substituted.

Some of these compounds, and methods for their preparation, were previously disclosed in WO-A-92/18614 although that document discloses only that the compounds act as autoinducers and as agents for the control of gene expression. Compounds in the same series are also mentioned in Journal of Bacteriology, volume 175, number 12, June 1993, pages 3856 to 3862 but again there is no teaching that they might have any effect outside micro-organisms.

PCT/GB01/01435 describes the use of homoserine lactone compounds for topical application for autoimmune diseases such as psoriasis. The preferred active compound is N-(3-oxododecanoyl)-homoserine lactone. The active compound is preferably formulated in an ointment, cream or lotion.

G. Papaccio, Diabetes Res. Clin. Pract. vol.13, no.1, 1991, pages 95-102 discloses the use of N-acetylhomocysteine thiolactone as an enhancer of superoxide dismutase in an attempt to increase protection against chemically induced diabetes.

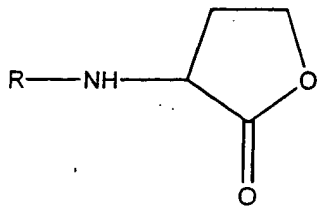
The use of N-acetylhomocysteine thiolactone to modify the IgE molecule is taught by J. Ljaljevic et al in Od. Med. Nauka, vol.24, 1971, pages 137-143 and Chemical Abstracts, vol.78, no.7, February 1973, abstract no. 41213a.

However, there is no suggestion in this paper of immunosuppression or of the inhibition of histamine release.

US-A-5,591,872 discloses the compound *N*-(3-oxododecanoyl) homoserine lactone as an autoinducer molecule. In "Infection and Immunity", vol.66, no.1, January 1998, the authors report the action of *N*-(3-oxododecanoyl)homoserine lactone (OdDHL) in inhibiting the concanavalin A mitogen stimulated proliferation of murine spleen cells and TNF-α production by LPS-stimulated adherent murine peritoneal macrophages.

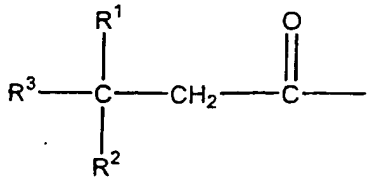
We have now discovered a subclass of N-acyl homoserine lactones that exhibits an immunosuppressant activity greater than that exhibited by similar compounds outside of this subclass.

According to one aspect, the present invention provides a compound of the formula I



(I)

in which R is an acyl group of the formula II



(II)

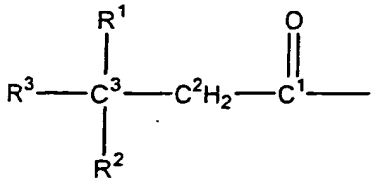
wherein one of R^1 and R^2 is H and the other is selected from OR^4, SR^4 and NHR^4, wherein R^4 is H or 1-6C alkyl, or R^1 and R^2 together with the carbon atom to which they are joined form a keto group, and R^3 is a straight or branched chain, saturated or unsaturated aliphatic hydrocarbyl group containing from 8 to 11 carbon atoms and is optionally substituted by one or more substituent groups selected from halo, 1-6C alkoxy, carboxy, 1-6C alkoxycarbonyl, carbamoyl optionally mono- or disubstituted at the N atom by 1-6C alkyl and NR^5R^6

wherein each of R^5 and R^6 is selected from H and 1-6C alkyl or R^5 and R^6 together with the N atom form a morpholino or piperazino group, or any enantiomer thereof, with the proviso that R is not a 3-oxododecanoyl group.

The compounds of the present invention are capable of modulating the immune response in the living animal body, including human: In particular, they have an inhibitory effect on lymphocyte proliferation in humans and down-regulate TNF-α secretion by monocytes/macrophages and, in consequence, the activation of Th1 lymphocytes in humans. The present invention, therefore, provides a pharmaceutical composition comprising a therapeutically-effective amount of a compound of the invention as described herein, including an enantiomer thereof, together with a pharmaceutically-acceptable carrier or diluent.

A further aspect of the invention provides the use of a compound of the invention, including an enantiomer thereof, for the manufacture of a medicament for the treatment of a disease of a living animal body including human which disease is responsive to the activity of an immunosuppressant, for example an autoimmune disease. A yet further aspect of the invention relates to a method of treating a disease of a living animal body, including a human, which disease is responsive to the activity of an immunosuppressant, e.g., an autoimmune disease, which method comprises administering to the living animal body, including human, a therapeutically-effective amount of a compound according to the invention, as described herein including an enantiomer thereof.

Compounds of the invention have the general formula I given above. The group R in the formula I has the formula II



(II)

In formula II according to a first preferred embodiment one of R^1 and R^2 is H and the other is selected from OR^4, SR^4 and NHR^4, in which R^4 is H or a 1-6C alkyl group. Preferably, R^4 is H. Such a definition of R^1 and R^2 gives rise to

chirality at the carbon atom to which R^1 and R^2 are attached (C-3). The compounds of the invention can, thus, be in the form of racemates, optically active isomers or mixtures thereof. According to a particular preferred embodiment one of R^1 and R^2 is H and the other is OH.

According to this first preferred embodiment the group R^3 in formula II is a straight or branched chain 8 to 11C aliphatic hydrocarbyl group which is saturated or which may be ethylenically unsaturated. The group may, further, be substituted by one or more substituent groups selected from halo, for example F, Cl, Br or I; 1-6C alkoxy, for example methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and tert-butoxy; carboxy including salts thereof, 1-6C alkoxycarbonyl, for example methoxycarbonyl, carbamoyl, for example N,N-dimethylcarbamoyl and NR^5R^6, wherein R^5 and R^6 are each selected from H and 1-6C alkyl or R^5 and R^6 together with the nitrogen atom to which they are attached form a morpholino group or a piperazino ring, optionally substituted at the 4-N by a methyl group. A particularly preferred R^3 group in formula II above is a straight chain or branched chain 8 to 11C alkyl group which is optionally substituted by one substituent selected from Br, carboxy including salts thereof, and methoxycarbonyl. The substituent is typically, though not necessarily, attached in a terminal position on the alkyl group. Alternatively, the R^3 group is a straight chain or branched chain 8-11C alkenyl group, preferably monoethenically unsaturated, which may be substituted by a substituent selected from Br, carboxy including a salt thereof, and methoxycarbonyl. Again, the substituent is typically, though not necessarily, attached in a terminal position on the alkenyl group.

In formula II above according to a second preferred embodiment the groups R^1 and R^2 together form an oxo group (=O) such that a keto group exists at the C-3 position in the acyl group. In such a case the group R^3 in formula II will typically be:

(a) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 8C aliphatic hydrocarbyl group;

(b) a substituted, saturated, straight or branched chain 9C aliphatic hydrocarbyl group;

10-methyl-3-oxoundecanoyl;

6-methyl-3-oxoundecanoyl;

3-hydroxydodecanoyl;

12-bromo-3-oxododecanoyl;

3-oxotridecanoyl;

13-bromo-3-oxododecanoyl;

3-hydroxytetradecanoyl;

3-oxotetradecanoyl;

14-bromo-3-oxotetradecanoyl; and

13-methoxycarbonyl-3-oxotridecanoyl.

Examples of acyl groups R of formula II above in which R^3 is an ethylenically unsaturated hydrocarbyl group include:-

3-oxo-12-tridecenoyl;

3-oxo-7-tetradecenoyl;

3-hydroxy-7-tetradecenoyl;

3-oxo-9-tetradecenoyl;

3-hydroxy-9-tetradecenoyl;

3-oxo-10-tetradecenoyl;

3-hydroxy-10-tetradecenoyl;

3-oxo-11-tetradecenoyl;

3-hydroxy-11-tetradecenoyl;

3-oxo-13-tetradecenoyl; and

3-hydroxy-13-tetradecenoyl.

The compounds of the present invention having the 3-oxo group may, in general, be prepared by a method comprising the steps of:

(1) reacting an acid having the general formula R^3COOH, where R^3 is as defined above, with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) in the presence of 4-dimethylaminopyridine and N,N^1-dicyclohexylcarbodiimide in a dry organic solvent, such as dry dichloromethane, to give the acylated Meldrum's acid; and

(c) an optionally-substituted, ethylenically-unsaturated, straight or branched chain 9C aliphatic hydrocarbyl group;

(d) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 10C aliphatic hydrocarbyl group; or

(e) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 11C aliphatic hydrocarbyl group.

In the case where the group R^3 is substituted, it will be substituted by one or more substituent groups selected from halo, for example F, Cl, Br or I; 1-6C alkoxy, for example methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and tert-butoxy; carboxy including salts thereof, 1-6C alkoxycarbonyl, for example methoxycarbonyl, carbamoyl, for example N,N-dimethylcarbamoyl, and NR^5R^6, wherein R^5 and R^6 are each selected from H and 1-6C alkyl or R^5 and R^6 together with the nitrogen atom to which they are attached form a morpholino group or a piperazino ring, optionally substituted at the 4-N by a methyl group.

According to one preferred embodiment the R^3 group in formula II above is a straight chain or branched chain 8, 10 or 11C alkyl group which is optionally substituted by one substituent selected from Br, carboxy including salts thereof, and methoxycarbonyl. According to another preferred embodiment the R^3 in formula II above is a straight chain or branched chain 9C alkyl group which is substituted by one substituent selected from Br, carboxy including salts thereof and methoxycarbonyl. The substituent is typically, though not necessarily, attached in a terminal position on the alkyl group.

According to yet another preferred embodiment the R^3 group is a straight chain or branched chain 8-11C alkenyl group, preferably monoethenically unsaturated, which may be substituted by a substituent selected from Br, carboxy including a salt thereof, and methoxycarbonyl. The substituent is typically, though not necessarily, attached in a terminal position on the alkyl group.

Examples of acyl groups R of formula II above in which R^3 is a saturated hydrocarbyl group include:-

3-oxoundecanoyl;

11-bromo-3-oxoundecanoyl;

(2) reacting the acylated Meldrum's acid with L-homoserine lactone hydrochloride in an organic solvent, e.g., acetonitrile, to give the *N*-(3-oxoacylated)-L-homoserine lactone.

Where the appropriate acid is not available it may be prepared by, for instance, oxidising the appropriate alcohol using chromic acid.

The *N*-(3-hydroxyacyl)-L-homoserine lactone may be prepared by reducing the corresponding *N*-(3-oxoacyl)-L-homoserine lactone using sodium cyanoborohydride in acid conditions.

As mentioned above, the compounds of the present invention have use as pharmaceutically active ingredients in the treatment of an animal body, including the human body, suffering from a disease or disorder which is responsive to the activity of an immunosuppressant, particularly for the treatment of type I diabetes mellitus (type 1A autoimmune). The dosage administered to the animal body in need of therapy will, of course, depend on the actual active compound used, the mode of treatment and the type of treatment desired as well as on the body mass. The active compound may, of course, be administered on its own or in the form of an appropriate medicinal composition containing, for instance, an appropriate pharmaceutical carrier or diluent. Other substances can, of course, also be employed in such medicinal compositions, such as antioxidants and stabilisers, the use of which is well known to persons skilled in the art.

Preferably, the compound is orally administered. The present inventors have found that, in the treatment of insulitis or type I diabetes mellitus, at present in NOD (non-obese diabetic) mice, that the compounds of the present invention have greater efficacy and bioavailability than the conventionally used compound vehicle DMSO. Furthermore, the compounds of the present invention perform at least comparably to accepted immune modulators such as CsA or antibodies to TNF without exerting overt immune toxicity.

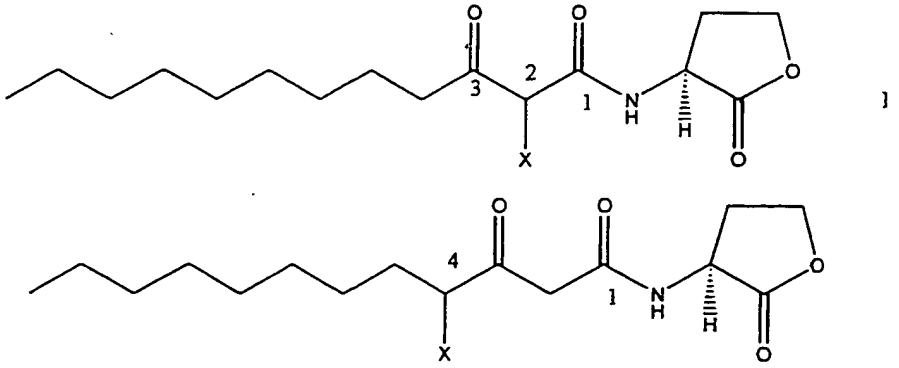
In comparative *in vitro* studies for the prevention of cell proliferation CSA and dexamethasone performed better than OdDHL. However, unexpectedly, when the assay was performed *in vivo* OdDHL performed better than CSA and dexamethasone (see below), leading the present inventors to conclude that in

an *in vivo* situation CSA and dexamethasone are either inactivated or prevented from acting by metabolic processes or by physiological breakdown of the compound whereas OdDHL is either resistant to these processes or changes or is somehow further activated by them. From this it is believed that a similar result will be achieved for insulitis.

The most preferred compound of the present invention is OdDHL[N-(3-oxododecanoyl)-L-homoserine lactone] and derivatives or substituents thereof, as set out in PCT/GB01/01453, the content of which is incorporated herein by reference.

In another aspect of the invention, the OdDHL or the other related immune modulatory compounds may be used in the identification of molecular targets and novel immunophilins in cells, preferably pancreatic beta cells or autoreactive leucocytes by constructing affinity matrices incorporating the compounds.

Two possible chemical strategies which may be employed in the production of such an affinity matrix are the synthesis of side-chain functionalised, for example C2 or C4-functionalised OdDHL (3O, C12-HSL) derivates (X = $(CH_2)_nCOOH$) as shown for side chains in Formulae I and II below. The length of the alkyl chain (n = 1, 2, 3 etc) will determine the efficiency of binding to the novel immunophilins.



I

II

In the above formulae, X may be selected from Br, Cl, I or (CH_2)nCOOH.

Alternatively, the molecule may be terminally functionalised following the schemes shown in Figures 13 and 14.

In a further aspect of the invention, more potent immune modulatory agents can be generated by the synthesis of bivalent OdDHL, PQS and hybrids. These bivalent ligands can be constructed by, linking PQS and HdDHL (3OH, C12-HSL) through a spacer of optimum length to provide a Homo-dimer (See Figure 1) or Hetero-dimer (See Figure 2).

Preferably, the two molecules will be linked though their respective 3-OH substituents via a spacer. Alternatively, for the Homo-dimers, when the C2 or C4 substituent is carboxyalkyl [$(CH_2)_nCOOH$], OdDHL-dimers can be synthesised by the method described in J.*Med. Chem.* **2001**, *44*, 1615-1622. Without wising to be bound by theory, the present inventors believe that the nature of the substituent at C2 or C4, that is the halogen or the carboxyalkyl will determine or limit the nature of the spacer linking the two molecules. For example, the choice of the spacer is likely to have an effect on the immunomodulatory properties of the dimer through possible sites of the linkage of the spacer and the nature of the covalent linkage. Additionally, the length of the spacer is important when determining the effectiveness of the cross-linking since it is desired to create a compound which exhibits a potency that is greater than that derived from the sum of its two monovalent pharmacophores. It is desirable to use a flexible or conformationally restricted spacer, especially in order to prevent adverse effect with steric hindrance. Some examples of spacers are shown in Figure 3.

Optionally, the carboxyalkyl substituent at C2 or C4 can be tagged (for example, with a colorimetric or fluorometric tag) for cell compartmentalisation assays or targeting studies, or simply for use in the affinity matrix studies, for example to show binding. This strategy of tagging via C2 or C4 is especially preferred as the biologically important parts of the molecule are unaffected or at least are still available for chemical, biochemical or physiological interactions.

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the following examples which are illustrated with reference to the accompanying drawings, of which

Figures 1, 2 and 3 have already been described;

Figure 4 is a graph showing the comparative *in vitro* anti proliferative effects of CSA, dexamethasone and OdDHL;

Figure 5 is a graph showing the influence of oral OdDHL on a murine *in vivo* DTH response to SRBCs;

Figure 6 is a graph showing the proliferation of Balb/C splenocytes stimulated with ConA. in the presence of OdDHL and OOHL;

Figure 7 is a graph showing the proliferation of human PBMC stimulated with ConA in the presence of OdDHL and OtDHL;

Figure 8 is a graph showing TNFα production by human PBMC stimulated with LPS in the presence of OdDHL and OtDHL;

Figure 9 is a graph showing TNF-α production in the presence of test compounds;

Figure 10 is a graph showing TNF-α production by human PBMC in the presence of test compounds;

Figure 11 is a graph showing TNFα production in the presence of drugs;

Figure 12 is a graph showing lack of overt toxicity of OdDHL.

Figure 13 shows schematically the synthesis of a terminally functionalised OdDHL; and

Figure 14 shows schematically the synthesis of a terminally functionalised OdDHL.

EXAMPLES

Example 1: N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL)

To a solution of decanoic acid (2mmol) in dry dichloromethane (20 ml) was added 4-dimethylaminopyridine (2.1 mmol), *N,N'*-dicyclohexylcarbodiimide (2.2 mmol) and Meldrum's acid (2 mmol). The solution was stirred at room temperature overnight and then filtered to remove the precipitated dicyclohexylurea. The filtrate was evaporated to dryness and the residue redissolved in ethyl acetate. The ethyl acetate solution was washed with 2 M hydrochloric acid, dried over anhydrous magnesium sulphate and concentrated to afford the decanoyl Meldrum's acid.

To a stirred solution of the decanoyl Meldrum's acid (1 mmol) in acetonitrile (30 ml) was added L-homoserine lactone hydrochloride (1 mmol) and triethylamine (1.2 mmol). The mixture was stirred for 2 hours and then refluxed for a further 3 hours. The solvent was removed by rotary evaporation to give a residue that was redissolved in ethyl acetate. The organic solution was sequentially washed with saturated sodium hydrogen carbonate solution, 1M potassium hydrogen sulphate solution and saturated sodium chloride solution. After drying over anhydrous magnesium sulphate, the organic extract was evaporated to dryness and the residue purified by preparative layer chromatography on silica plates.

^{1}H NMR (250 MHz, $CDCl_3$) δ 0.9 (3H, t, CH_3), 1.27 (12H, m, $CH_3(CH_2)_6$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH).

The procedure described above in Example 1 was followed to prepare other *N*-(3-oxoacylated)-L-homoserine lactones as described below using, in each case, the appropriate carboxylic acid.

Example 2: *N*-(12-Bromo-3-oxododecanoyl)-L-homoserine lactone (12BrOdDHL)

^{1}H NMR (250 MHz, $CDCl_3$) δ 1.27 (10H, m, $BrCH_2CH_2(CH_2)_5$), 1.45 (2H, m, $BrCH_2CH_2CH_2$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 3.53 (2H, t, $BrCH_2$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH).

Example 3: *N*-(12-Hydroxy-3-oxododecanoyl)-L-homoserine lactone (12OHOdDHL)

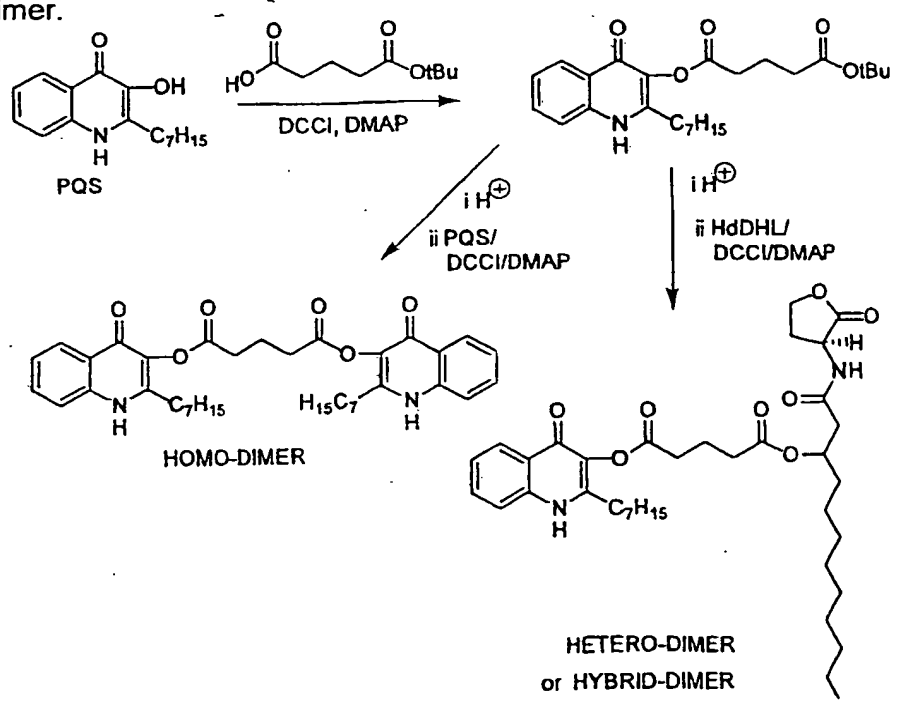
Using 10-acetoxydecanoic acid in the general procedure as described above in Example 1 afforded the *N*-(12-acetoxy-3-oxododecanoyl)-L-

homoserine lactone. The latter when refluxed in 1M hydrochloric acid, yielded the title product.

^{1}H NMR (250 MHz, $CDCl_3$) δ 1.27 (12H, m, $HOCH_2(CH_2)_6$), 1.59 (2H, m, CH_2CH_2CO), 1.89 (1H, t, OH), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 3.60 (2H, t, $HOCH_2$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH).

Example 4: Synthesis of a homo and a hybrid-dimer by linking through 3-OH substituents

N,N'-Dicyclohexylcarbodiimide (DCCI) and 4-dimethylaminopyridine (DMAP) catalysed acylation of the 3-OH substituent of the PQS with glutaric acid mono t-butyl ester would furnish the acylated PQS (Scheme 1). Removal of the tBu protection by acidolysis is followed by esterification of the resultant carboxylic acid either with the 3-OH substituent of another molecule ofPQS to furnish the homo-dimer or HdDHL (3-OH, C12-HSL) to deliver the desired hetero-dimer.



Scheme 1: Synthesis of a homo- and hetero-dimer by linking through 3-OH substituents.

Example 5: Effect of OdDHL on insulitis

To investigate the effect of OdDHL on insulitis or type I diabetes mellitus, mice (NOD) with a genetic predisposition to develop IDDM (insulin dependent diabetes mellitus) were treated with OdDHL.

The animals were dosed at 100mg/kg intraperitoneally from 4 weeks of age, 3 times a week for four weeks. Insulitis was assessed at 14 weeks.

In the table a score of 3 or more (DMSO and PBS) represents severe grade insulitis and 1.3 (OdDHL) represents little infiltration (a score of 1 would represent low grade peri-insulitis).

The results for 100mg/kg are shown in Table 1 below. The mice were scored according to the method of Beales *et al* (European Journal of Pharmacology 357(1998) 221-225).

Table 1

Animal Number	DMSO Slide Score	PBS Slide Score	OdDHL Slide Score
1	3.160		
2			1.000
3			1.418
4		3.372	
5		1.891	
6	3.855		
7	4.352		
8		3.937	
9			1.936
10	3.202		
11			1.478
12		2.690	
13		3.806	
14	1.735		
15		3.184	
16			1.154
17	2.019		
Group Total	18.23	18.880	6.986
Group Mean	3.054	3.140	1.397
Standard Derivation	1.01691	0.76115	0.35853

Example 6: Oral availability of OdDHL

To show that OdDHL is orally available, the anti-sheep red blood cell (SRBC) responses of mice were measured in accordance with the following experiment. Eight week old, female Balb/c mice were kept on a 12 hour light/dark cycle and fed food and water *ad libitum.* SRBCs in Alseviers solution (TCS Biologicals, SB069) were washed and centrifuged (800 x g for 20 minutes

at room temperature) three times with 0.9% NaCl in distilled water. Cells were counted using a haemocytometer. On day 0 animals were immunised intraperitoneally (IP) with 5 $x10^6$ (low dose) or 5 x 10^8 (high dose) SRBCs in saline. On day 5, animals were challenged with 20µl SRBCs at 5 x 10^9/ml in the left hind paw. The contralateral paw was injected with 20 µl saline alone. After 24 hours the animals were sacrificed with a rising concentration of CO_2 and bled by cardiac puncture. The hind feet were severed at the ankle joint and weighed.

Animals were dosed po from day 0 to day 5 with OdDHL. OdDHL was suspended in 0.25% (wt/vol) cellosize (Boots Co. PLC, Nottingham UK) in distilled water containing 1.5% (vol/vol) Tween 80 (Registered Trade Mark, Sigma) for po dosing at 3, 10, 30 and 100mg/kg. Animals received 0.1 ml po.

It was found that immunisation of Balb/c mice with 5 x 10^6 SRBCs evokes a delayed type hypersensitivity response (type IV) when challenged with SRBCs in the footpad. This manifests itself as an inflammatory response causing an increase in the size of the foot. Very little antibody is produced. OdDHL at 3, 10, 30 and 100 mg/kg caused a dose dependent increase in footweight of 37%, 60%, 100% and 93% respectively (see Figure 5). Mice dosed with OdDHL had higher footweights when compared to vehicle dosed animals. The 30 and 100 mg/kg doses (** on graph) are significant as determined by Dunnett's multiple comparison's test after one-way analysis of variance ($p<0.01$). This assay is considered by the pharmaceutical industry to be Th2 dependent, indicating the effect of compound treatment on contra-regulating T-helper 1 lymphocytes.

Example 7: Immunomodulatory Activity of Homoserine Lactone Compounds

Materials and Methods

I. ConA mitogen-stimulated proliferation of murine splenocytes

The concanavalin A (ConA) cell proliferation assay was used to assess the effect of homoserine lactone (HSL) compounds on T-cell activation and proliferation. Proliferation was assessed by the incorporation of [^{3}H]-thymidine into DNA. Eight-week-old female BALB/c mice were obtained from Harlan

(Bicester, Oxon, UK) and given food and water *ad libitum*. Splenocyte suspensions were prepared by removing the spleens and placing them into RPMI 1640 medium. The spleens were forced through 70-μm-pore-size wire gauzes using the plunger from a 5-ml syringe to produce a single cell suspension. The cells were pelleted by centrifugation, and erythrocytes were lysed with 0.017M Tris, 0.144M ammonium chloride buffer, pH 7.2. Leucocytes were washed twice with RPMI 1640 medium with 2% (vol/vol) foetal calf serum (FCS) and resuspended in complete cell culture medium (CTCM) consisting of RPMI 1640 medium with 5% FCS, 2mM L-glutamine, and 5 x 10^{-5} M 2-mercaptoethanol. HSL compounds were tested at doubling down dilutions ranging from 1 mM to 0.1 μM in a final volume of 200 μl of CTCM, containing ConA (Sigma, Poole, UK) at 1 μg/ml and 100,000 spleen cells. Following incubation for 48 h at 37°C in 5% CO_2-air, 0.25 μCi [^{3}H]-thymidine (Amersham) in 10 μl volume made up in RPMI 1640 medium was added and the cells were incubated for a further 24 h. Cells were harvested onto fibreglass filters with a Packard filtermate harvester. After the addition of 25 μl of MicroScint-O (Packard) to each well the filters were counted with the Packard TopCount scintillation counter.

Mitogen (Concanavalin A) induced murine splenocyte proliferation was indicated by the incorporation of tritated thymidine into the DNA in the mouse spleen cells as shown by counts per minute using the scintillation counter. The inhibitory effect of an HSL compound being tested on cell proliferation was indicated by a reduction in counts per minute. Figure 6 shows the plots of counts per minute (cpm) against the concentrations (micromolar) of the HSL compounds *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) and *N*-(3-oxooctanoyl)-L-homoserine lactone (OOHL) and the vehicle dimethylsulphoxide (DMSO). It can be seen, from this figure, that OdDHL inhibits splenocyte proliferation. In contrast, OOHL and DMSO failed to inhibit proliferation.

The IC50 value, i.e., the concentration (micromolar) of a compound which inhibits cell proliferation thymidine incorporation by 50% was determined for

several compounds of the present invention and these IC 50 values are shown in column A of the Table below.

II. ConA mitogen-stimulated proliferation of human PBMC

Blood specimens were obtained with consent from healthy human volunteers. Human peripheral blood mononuclear cells (PBMC) were isolated from heparinised whole blood by buoyant density centrifugation over Histopaque 1077 (Sigma, Poole, UK) at 600g for 20 minutes. PBMC harvested from the 'buffy' layers were washed twice with RPMI 1640 medium and resuspended in CTCM. HSL compounds were tested at similar dilutions as for murine splenocytes in 200 μl of CTCM, containing 1 μg/ml of ConA and 100,000 PBMC. Human PBMC were incubated for 48 h at 37°C in 5% CO_2-air, followed by pulsing with 0.25 μCi [^{3}H]-thymidine (see above). After a further incubation of 24 h cells were harvested onto fibreglass filters and then counted in the presence of MicroScint-O with the Packard TopCount.

Concanavalin induced cell proliferation of human peripheral blood mononuclear cells (PBMC) was tracked, as described in I above, by a measurement of counts per minute using the scintillation counter. The inhibitory effect of an HSL compound being tested on cell proliferation was indicated by a reduction in counts per minute. Figure 7 shows the plots of cpm against the concentrations of OdDHL, *N*-(3-oxotetradecanoyl)-L-homoserine lactone (OtDHL) and DMSO (vehicle). As can be seen, both OdDHL and OtDHL inhibited proliferation of human PBMC stimulated with Concanavalin A.

The IC50 values for several HSL compounds of the invention were determined and these are shown in columns B, C and D in the Table below. Columns B, C and D represent different sources of human PBMC samples used.

III. TNF-alpha production from LPS-stimulated human PBMC

Bacterial lipopolysaccharide (LPS) stimulates the production of a variety of cytokines, including TNF-alpha, from human PBMC; these cytokines in turn influence the development of T cells, supporting a T helper 1 conducive milieu.

Human PBMC prepared from whole blood by buoyant density centrifugation were resuspended in CTCM. HSL compounds were again tested at similar dilutions as for murine splenocytes in 200 µl of CTCM, containing 5 x 10^{-5} µg/ml LPS *Escherichia coli* strain 055:B5 (Sigma, Poole, UK) and 100,000 PBMC. Following incubation for 24 h at 37°C in 5% CO_2-air, the cell culture supernatants were collected and tested for TNF-alpha levels by 'sandwich' ELISA. Briefly, 96-well Nunc MaxiSorp (Life Technologies, Paisley, UK) plates were coated with 50 µl of a 2 µg/ml solution of mouse anti-human TNF-alpha monoclonal antibody (Pharmingen, UK) in 0.05 M carbonate/bicarbonate buffer, pH 9.6 overnight at 4°C. After washing the plates three times with PBS-Tween, which contained phosphate buffered saline (PBS) with 0.5% (vol/vol) Tween 20 (Sigma, Poole, UK), the plates were blocked with 1% (wt/vol) bovine serum albumin (BSA) (Sigma, Poole, UK) at room temperature for 2 h. Following three washes with PBS-Tween, 50 µl of cell culture supernatants were added and incubated overnight at 4°C; standard human TNF-alpha (Pharmingen, UK) ranging from 2000 to 31.25 pg/ml were included for each plate. After four washes with PBS-Tween, 50 µl of a second antibody, biotinylated mouse anti-human TNF-alpha monoclonal antibody (Pharmingen, UK) was added at 0.5 µg/ml diluted in 1% BSA in PBS-Tween and incubated at room temperature for 1 h. Following four washes, the bound biotinylated antibody was detected with 50 µl of a 1:1,000 dilution of Streptavidin-peroxidase (Pharmingen, UK). At the end of an hour incubation at room temperature, the plates were thoroughly washed six times with PBS-Tween and the assay was developed by the addition of 100 µl of 0.1 mg/ml of tetramethyl benzidine subtrate (Sigma, Poole, UK) in 0.1 M sodium acetate buffer, pH 6 containing 0.03% H_2O_2. The enzyme reaction was stopped with 50 µl of 2.5 M H_2SO_4 after an incubation of 10 minutes at room temperature and the development was read at 450 nm with a spectrophotometric 96-well plate reader (Dynex).

The effect of the concentration of certain HSL compounds of the invention on LPS induced TNF-α production by human PBMC was observed. Figure 8 shows plots of TNF-α concentrations (pg/ml) against the concentration

(micromolar) of OdDHL, OtDHL and DMSO (vehicle). As can be seen, both OdDHL and OtDHL inhibited the secretion of the T helper 1-supporting cytokine TNF-α. The IC50 values, i.e., the concentration (micromolar) of a compound which inhibits TNF-α secretion by 50%, was determined for some of the HSL compounds of the invention and these are shown in column E in the Table below.

Similar studies were carried out using, as the HSL compounds, N-(12-bromo-3-oxododecanoyl)-L-homoserine lactone (12BrOdDHL) and N-(12-hydroxy-3-oxododecanoyl)-L-homoserine lactone (12hydroxyOdDHL) and the plots for these are shown in Figure 9. For comparison purposes, similar studies were carried out using, as the HSL, the known shorter side chain compound N-(3-oxohexanoyl)-L-homoserine lactone (OHHL) and the plot for this is shown in Figure 10. The difference in activity between OHHL and OdDHL is marked. Also for comparison purposes, similar studies were carried out using the known drugs dexamethasone and Cyclosporin A (CsA) and the plots for these are shown in Figure 6. The IC50 value for dexamethasone was determined to be 500.

IV. Optimisation of cell culture conditions

In the cell culture assays the number of cells used (mouse splenocytes and human PBMC) was initially optimised to 100,000 cells per well. The optimal dose of ConA of 1 μg/ml used in the cell proliferation assays was determined from ConA titration curves. A similar titration curve was established for LPS stimulation to obtain an LPS concentration which stimulated a suboptimal level of TNF-alpha release from human PBMC.

Example 8: Comparative Immune Toxicity Assessment

The overt immune toxicity of OdDHL was investigated using the dose regime described above in Example 5 for the alleviation of insulitis or diabetes in NOD mice. Treated mice gained weight identically to their non-treated littermates. The results are shown in Figure 12. Splenocytes from the mice were taken along the time course of treatment (3 times a week for 4 weeks) and

were stained with anti-CD-3 (pan T-cell), anti-CD4 (helper T-cell), anti-CD8 (cytotoxic T-cell) and anti-CD19 (B cell) antibodies for 30 minutes on ice. Cells were washed twice in PBS/BSA and then fixed in 0.5% formaldehyde. Cell phenotypes were analysed on BD FACScan. As can be seen from Figure 12, a lack of overt immune toxicology was displayed during treatment with the immune cell populations remaining constant in proportion throughout the course of treatment.

Example 9: *in vivo* Effects of CSA. Anti-TNFα and OdDHL

Dose regimes of Cyclosporin A, anti-TNFα antibody and OdDHL were compared in the diabetes model. NOD mice were dosed at 25 mg/kg on alternate days for 160 days following the methodology of Mori *et al* (Diabetologia (1986) 29: 244-247), the content of which is incorporated herein by reference, for the investigation of CSA, and Anti TNF at 12 mg per individual, 0.5 mg three times a week for 8 weeks in accordance with the method of Suk *et al* (J. Immunology (2001) 166: 4481-4489), the content of which is incorporated herein by reference, was used for TNFα. OdDHL was dosed at 30 mg/kg, 3 times per week for 4 weeks as above. When compared to the reported data of Mori and Suk (*Supra*) OdDHL displayed more effective *in vivo* action than *in vitro* contrary to what would be expected from the *in vitro* results previously discussed (page 8).

Example 10: Confirmation of alleviation of diabetes in NOD mice

The ability of the lead compound (OdDHL) to alleviate diabetes in NOD mice was determined by treating the mice 3 times per week for 4 weeks at 30 mg/kg as above. DMSO and OHHL were used as controls.

Diabetes was diagnosed using Uristix and a level of above 6mMol glucose/l was taken to indicate the presence of diabetes. The results are shown in Table 2, which shows the incidence of diabetes at significant weeks of the experiment.

Table 2

Week of Experiment	20	30
DMSO	7/12	15/20
OHHL	7/10	12/20
OdDHL	0/11	5/20

A Kaplan-Meier analysis of cumulative incidence of diabetes shows:-

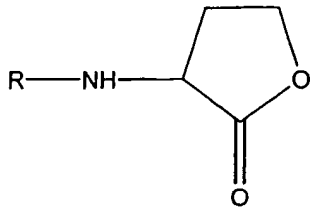
OdDHL vs DMSO p = 0.0004

OdDHL vs OHHL p = 0.009

DMSO vs OHHL = non significant (NS)

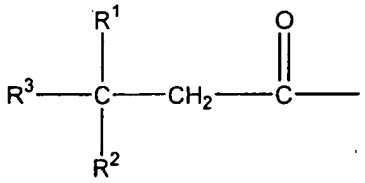
CLAIMS

1. An immune response modulatory compound of the formula I



(I)

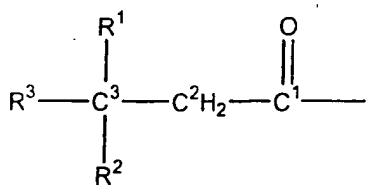
in which R is an acyl group of the formula II



(II)

wherein one of R^1 and R^2 is H and the other is selected from OR^4, SR^4 and NHR^4, wherein R^4 is H or 1-6C alkyl, or R^1 and R^2 together with the carbon atom to which they are joined form a keto group, and R^3 is a straight or branched chain, saturated or unsaturated aliphatic hydrocarbyl group containing from 8 to 11 carbon atoms and is optionally substituted by one or more substituent groups selected from halo, 1-6C alkoxy, carboxy, 1-6C alkoxycarbonyl, carbamoyl optionally mono- or disubstituted at the N atom by 1-6C alkyl and NR^5R^6 wherein each of R^5 and R^6 is selected from H and 1-6C alkyl or R^5 and R^6 together with the N atom form a morpholino or piperazino group, or any enantiomer thereof, with the proviso that R is not a 3-oxododecanoyl group.

2. A compound according to Claim 1, in which group R in the formula I has the formula II



(II)

in which one of R^1 and R^2 is H and the other is selected from OR^4, SR^4 and NHR^4, in which R^4 is H or a 1-6C alkyl group.

3. A compound according to claim 2, in which R^4 is H.

4. A compound according to Claim 3, in which one of R^1 and R^2 is H and the other is OH.

5. A compound according to any one of claims 1 to 4, in which R^3 of formula II is a straight or branched chain 8 to 11C aliphatic hydrocarbyl group which is saturated or ethylenically unsaturated.

6. A compound according to Claim 5, in which the R^3 group may be further substituted by one or more substituent groups selected from halo, F, Cl, Br or I; 1-6C alkoxy, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and tert-butoxy; carboxy including salts thereof, 1-6C alkoxycarbonyl, methoxycarbonyl, carbamoyl, N,N-dimethylcarbamoyl and NR^5R^6, wherein R^5 and R^6 are each selected from H and 1-6C alkyl or R^5 and R^6 together with the nitrogen atom to which they are attached form a morpholino group or a piperazino ring, optionally substituted at the 4-N by a methyl group.

7. A compound according to Claim 5, in which R^3 group in formula II is a straight chain or branched chain 8 to 11C alkyl group which is optionally substituted by one substituent selected from Br, carboxy including salts thereof, and methoxycarbonyl.

8. A compound according to Claim 5, in which R^3 group in formula II is a straight chain or branched chain 8-11C alkenyl group, preferably monoethenically unsaturated, which may be substituted by a substituent selected from Br, carboxy including a salt thereof, and methoxycarbonyl.

9. A compound according to any one of Claims 1 to 8, in which the groups R^1 and R^2 together form an oxo group (=O) such that a keto group exists at the C-3 position in the acyl group.

10. A compound according to Claim 9, in which the group R^3 in formula II will typically be:

(a) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 8C aliphatic hydrocarbyl group;

(b) a substituted, saturated, straight or branched chain 9C aliphatic hydrocarbyl group;

(c) an optionally-substituted, ethylenically-unsaturated, straight or branched chain 9C aliphatic hydrocarbyl group;

(d) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 10C aliphatic hydrocarbyl group; or

(e) an optionally-substituted, saturated or ethylenically-unsaturated, straight or branched chain 11C aliphatic hydrocarbyl group, and

in the case where the group R^3 is substituted, it is substituted by one or more substituent groups selected from the group consisting of halo, F, Cl, Br or I; 1-6C alkoxy, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, iso-butoxy and tert-butoxy; carboxy including salts thereof, 1-

6C alkoxycarbonyl, methoxycarbonyl, carbamoyl, N,N-dimethylcarbamoyl, and NR^5R^6, wherein R^5 and R^6 are each selected from H and 1-6C alkyl or R^5 and R^6 together with the nitrogen atom to which they are attached form a morpholino group or a piperazino ring, optionally substituted at the 4-N by a methyl group.

11. A compound according to claim 7 or claim 8, in which the substituent is attached in a terminal position on the alkyl group.

12. A compound according to any preceding claim, in which the acyl groups R of formula II in which R^3 is a saturated hydrocarbyl group include:-

3-oxoundecanoyl;
11-bromo-3-oxoundecanoyl;
10-methyl-3-oxoundecanoyl;
6-methyl-3-oxoundecanoyl;
3-hydroxydodecanoyl;
12-bromo-3-oxododecanoyl;
3-oxotridecanoyl;
13-bromo-3-oxododecanoyl;
3-hydroxytetradecanoyl;
3-oxotetradecanoyl;
14-bromo-3-oxotetradecanoyl; and
13-methoxycarbonyl-3-oxotridecanoyl.

13. A compound according to any preceding claim, in which the acyl groups R of formula II in which R^3 is an ethylenically unsaturated hydrocarbyl group include:-

3-oxo-12-tridecenoyl;
3-oxo-7-tetradecenoyl;
3-hydroxy-7-tetradecenoyl;
3-oxo-9-tetradecenoyl;

3-hydroxy-9-tetradecenoyl;
3-oxo-10-tetradecenoyl;
3-hydroxy-10-tetradecenoyl;
3-oxo-11-tetradecenoyl;
3-hydroxy-11-tetradecenoyl;
3-oxo-13-tetradecenoyl; and
3-hydroxy-13-tetradecenoyl.

14. Use of a compound according to any one of Claims 1 to 13 in a medicament for the modulation of immune response in the animal body.

15. Use of a compound according any one of Claims 1 to 13, in which the animal is a mammal.

16. Use of a compound according to any one of Claims 1 to 13, in which the mammal is a human.

17. Use of the compound of any one of Claims 1 to 13 in the manufacture of a medicament for the inhibition of lymphocyte proliferation.

18. Use of the compound of any one of Claims 1 to 13 in the manufacture of a medicament for the down-regulation of TNF-□ secretion by monocytes/macrophages and the consequent activation of Th 1 lymphocytes in humans.

19. A pharmaceutical composition comprising a therapeutically-effective amount of the compound according to any one of Claims 1 to 13 or an enantiomer thereof.

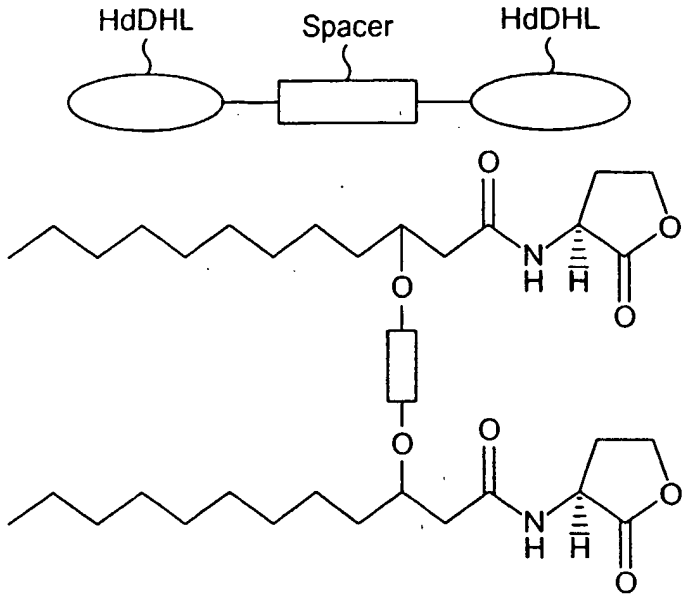
20. Use of the compound of any one of Claims 1 to 13 including enantiomers thereof, for the manufacture of a medicament for the treatment of a disease of a living animal body including human which disease is responsive to the activity of an immunosuppressant.

21. Use according to Claim 20, in which the disease is an autoimmune disease.

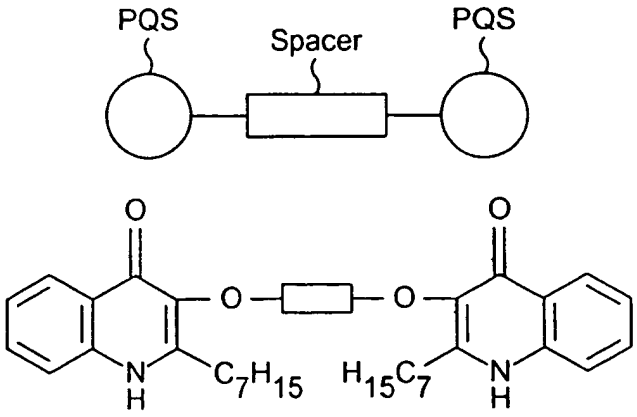
22. A method of treating a disease of a living animal body, including a human, which disease is responsive to the activity of an immunosuppressant, e.g., an autoimmune disease, which method comprises administering to the living animal body, including human, a therapeutically-effective amount of a compound according to Claim 1.

23. Use of compound according to any one of Claims 1 to 13, in which the compound is orally administered.

HOMO-DIMER 1



HOMO-DIMER 2



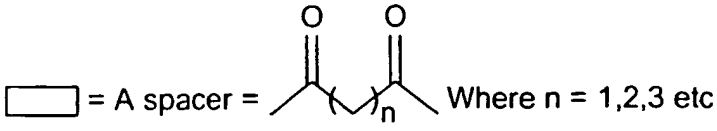


FIG. 1

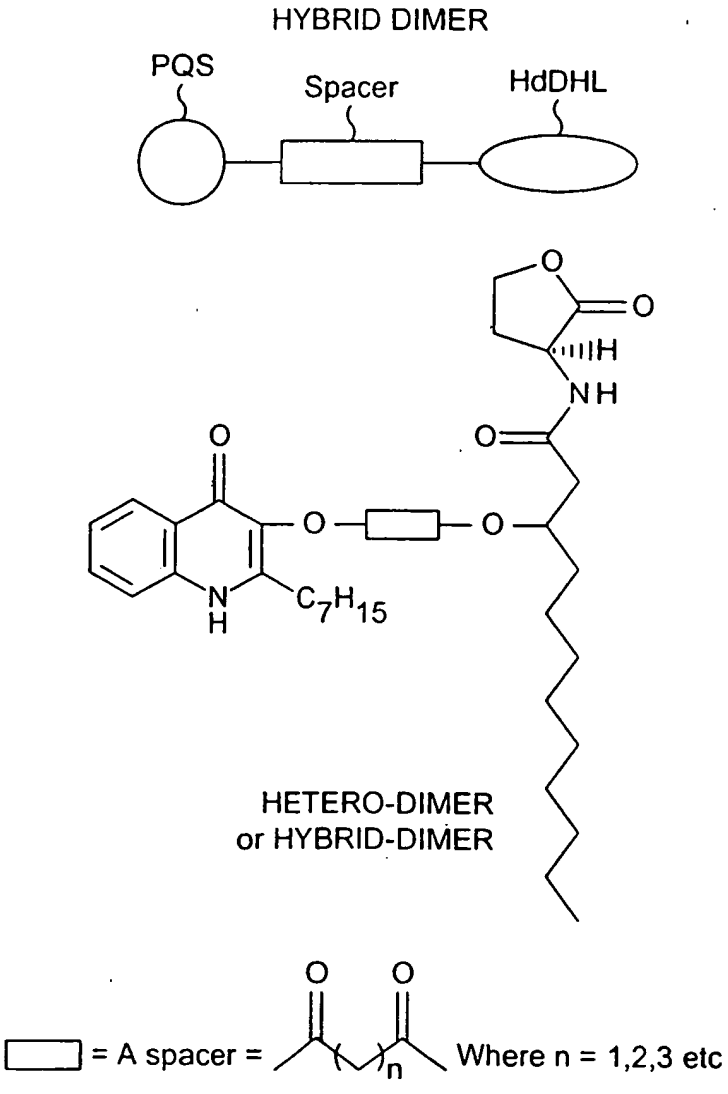
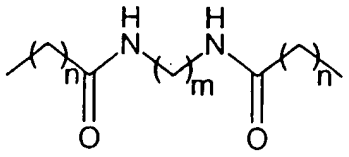
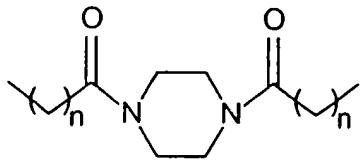
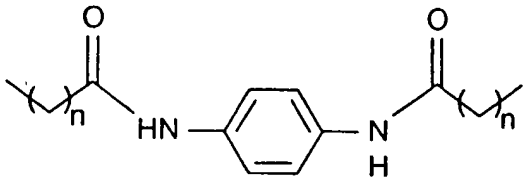

HYBRID DIMER
PQS
Spacer
HdDHL
O
O
H
NH
O
O
O
O
N
H
C_7H_{15}
HETERO-DIMER
or HYBRID-DIMER
= A spacer =
O
O
n
Where n = 1,2,3 etc

FIG. 2

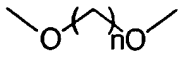
When X = $(CH_2)nCOOH$

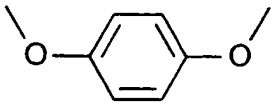
1.  flexible

2.  conformationally restricted

3. 

When X = Br

4. 

5. 

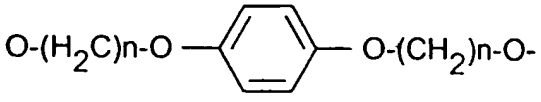
6. 

FIG. 3

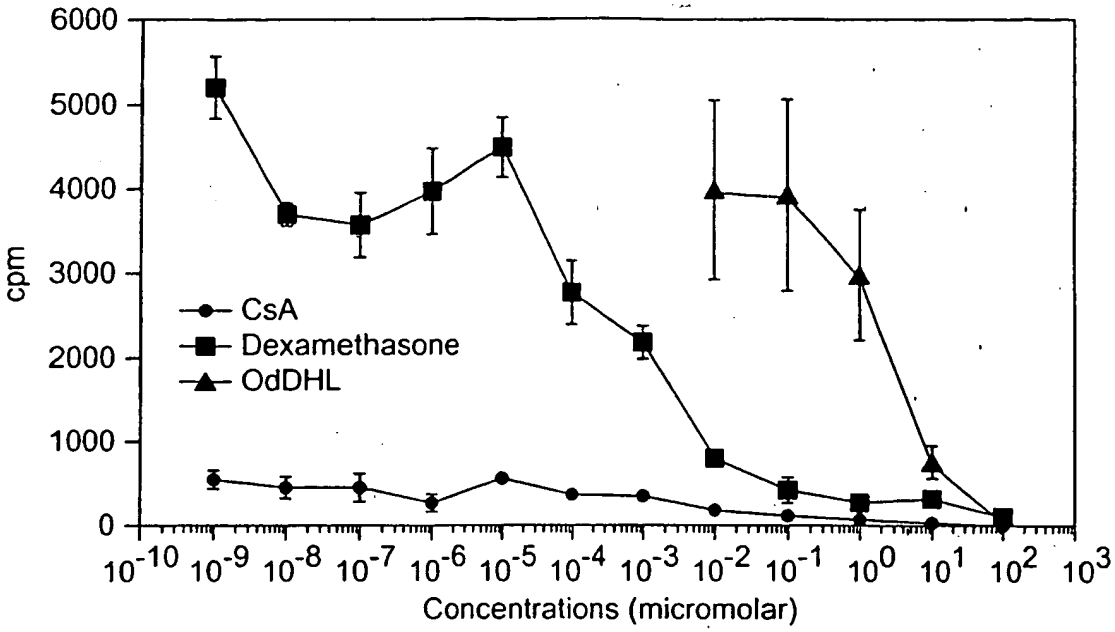

6000
5000
4000
3000
2000
1000
0
cpm
CsA
Dexamethasone
OdDHL
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Concentrations (micromolar)

FIG. 4

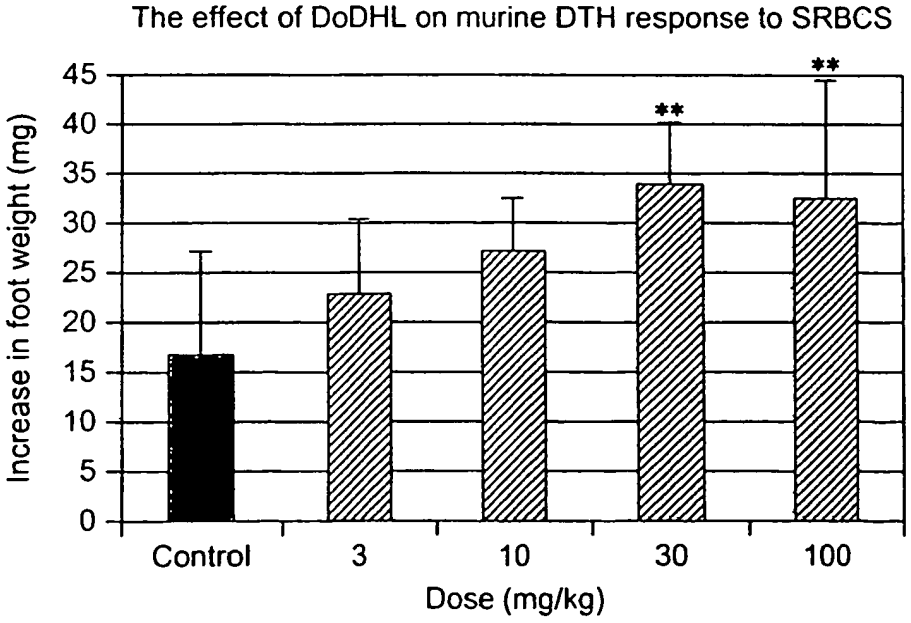

The effect of DoDHL on murine DTH response to SRBCS
Increase in foot weight (mg)
45
40
35
30
25
20
15
10
5
0
**
**
Control
3
10
30
100
Dose (mg/kg)

FIG. 5

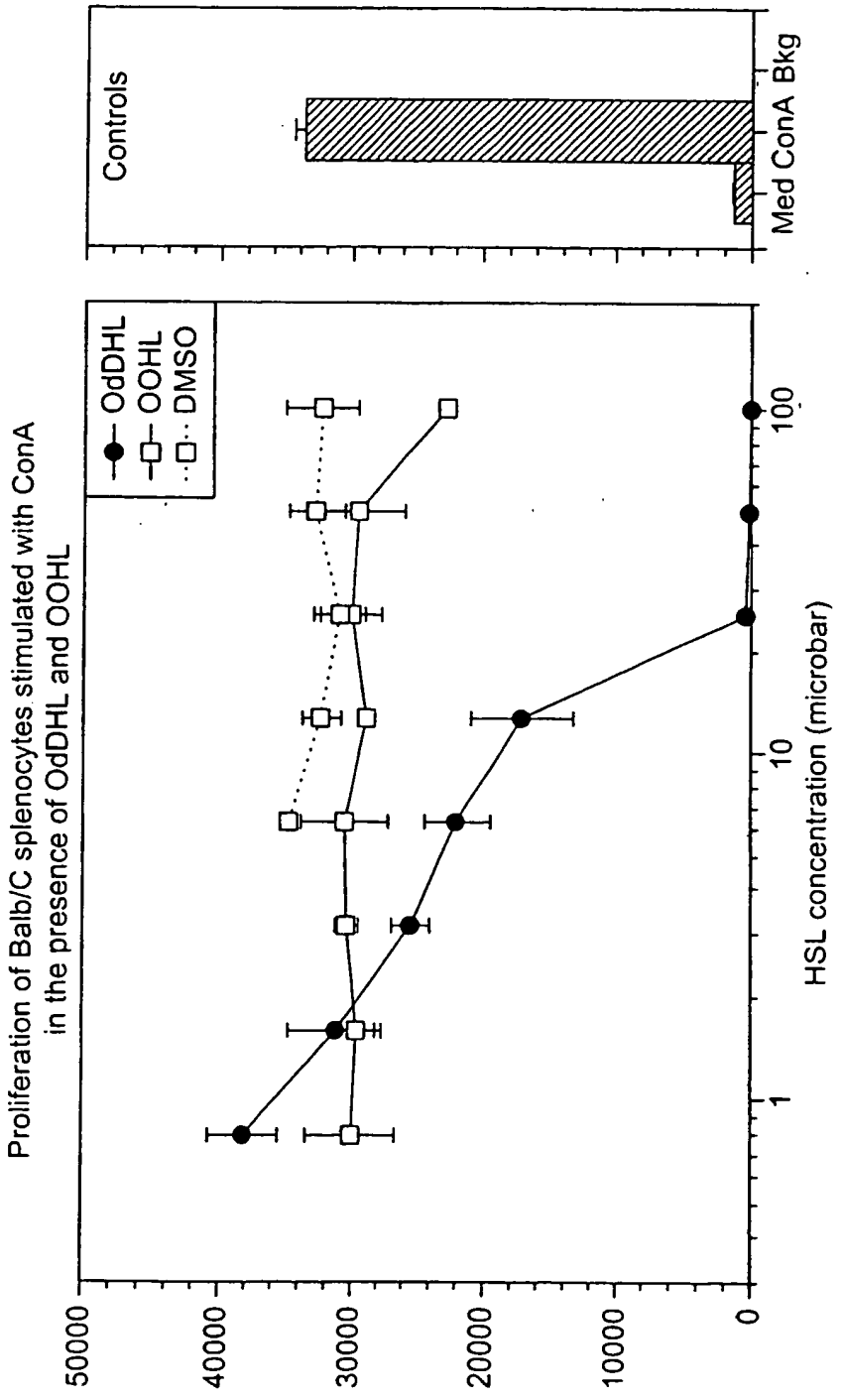

Controls
Med ConA Bkg
OdDHL
OOHL
DMSO
Proliferation of Balb/C splenocytes stimulated with ConA in the presence of OdDHL and OOHL
50000
40000
30000
20000
10000
0
1
10
100
HSL concentration (microbar)

FIG. 6

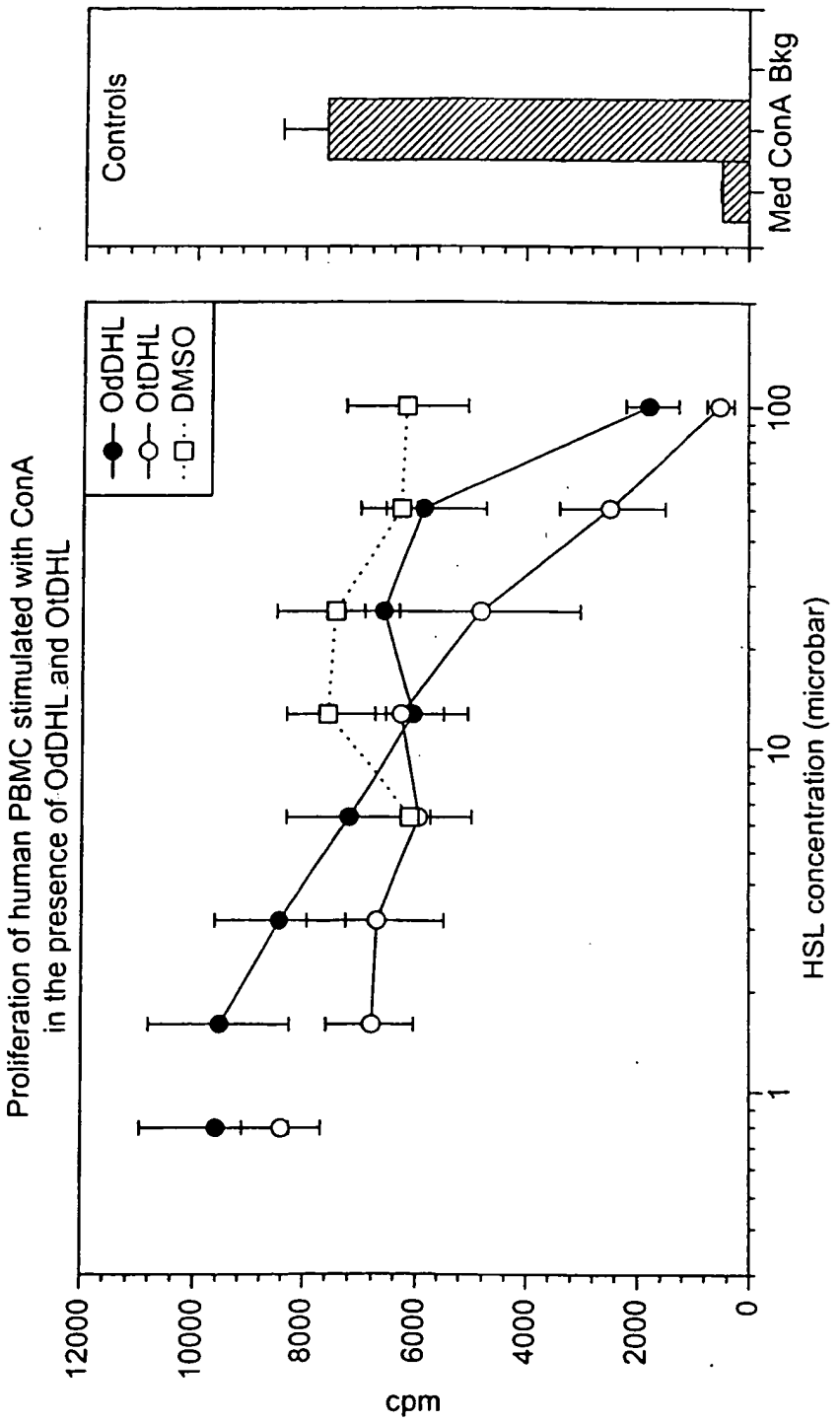

Controls
Med
ConA Bkg
Proliferation of human PBMC stimulated with ConA
in the presence of OdDHL and OtDHL
OdDHL
OtDHL
DMSO
cpm
12000
10000
8000
6000
4000
2000
0
1
10
100
HSL concentration (microbar)

FIG. 7

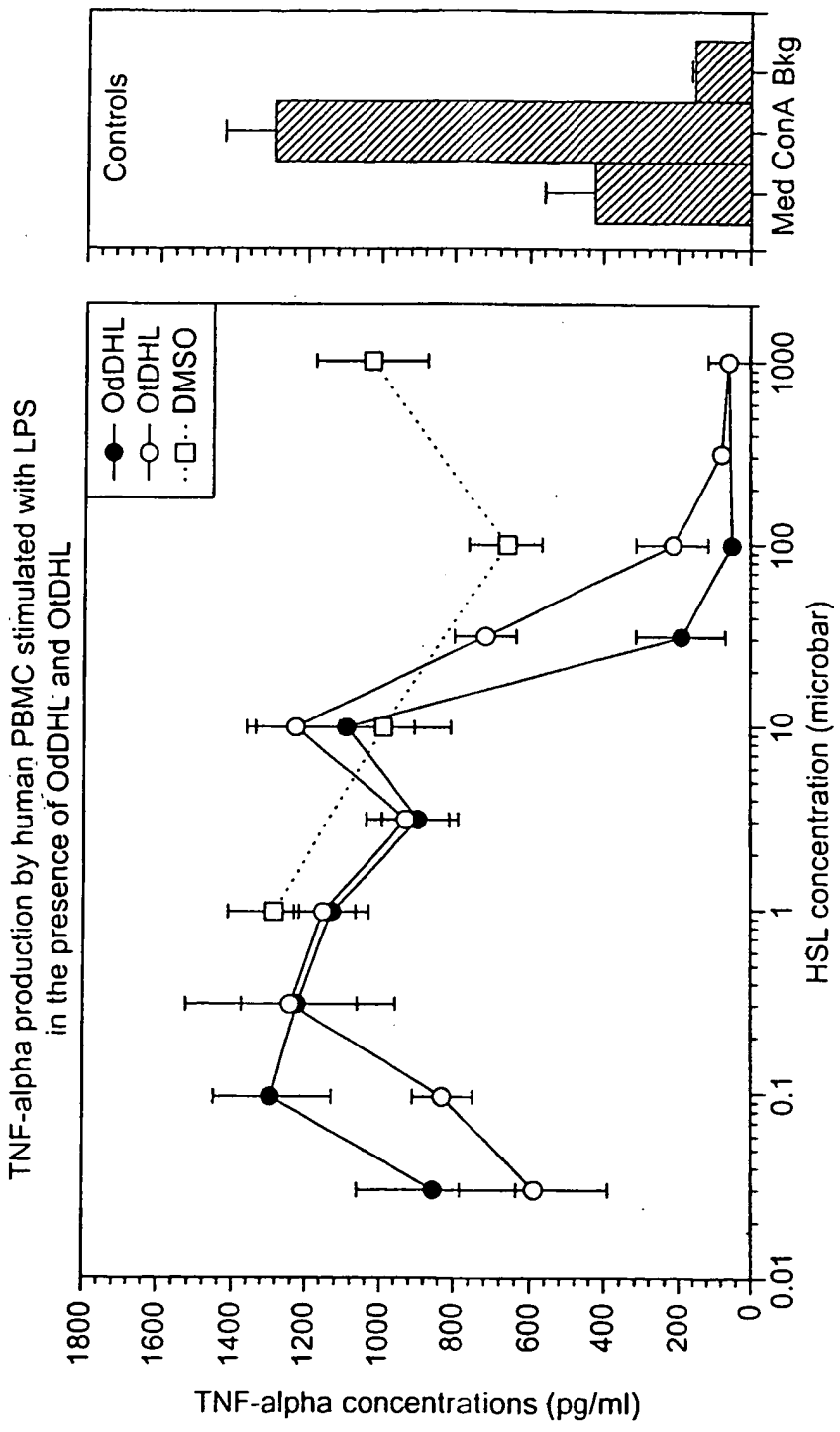

TNF-alpha production by human PBMC stimulated with LPS
in the presence of OdDHL and OtDHL
OdDHL
OtDHL
DMSO
TNF-alpha concentrations (pg/ml)
0
200
400
600
800
1000
1200
1400
1600
1800
HSL concentration (microbar)
0.01
0.1
1
10
100
1000
Controls
Med
ConA
Bkg

FIG. 8

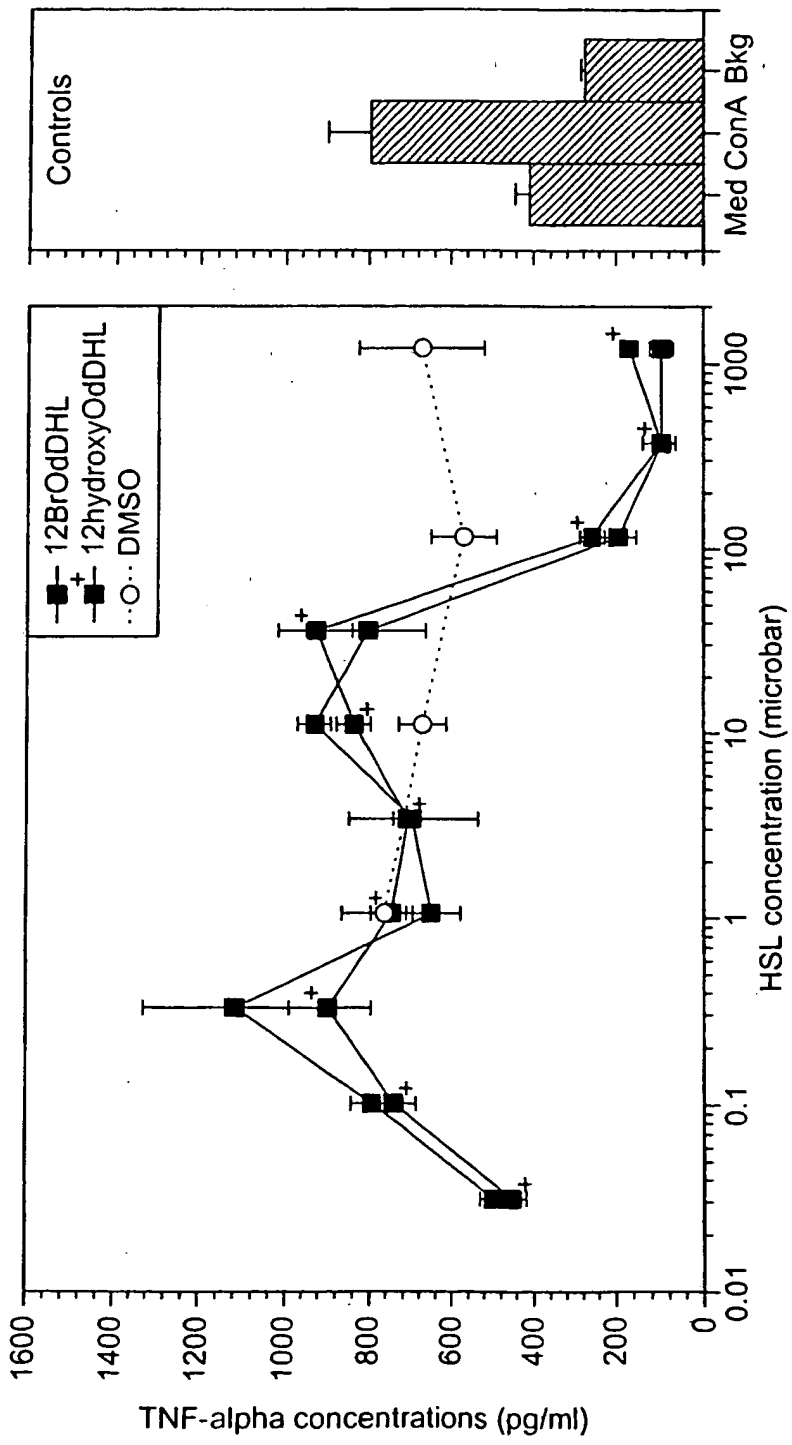

Controls
Med ConA Bkg
12BrOdDHL
12hydroxyOdDHL
DMSO
HSL concentration (microbar)
0.01
0.1
1
10
100
1000
TNF-alpha concentrations (pg/ml)
0
200
400
600
800
1000
1200
1400
1600

FIG. 9

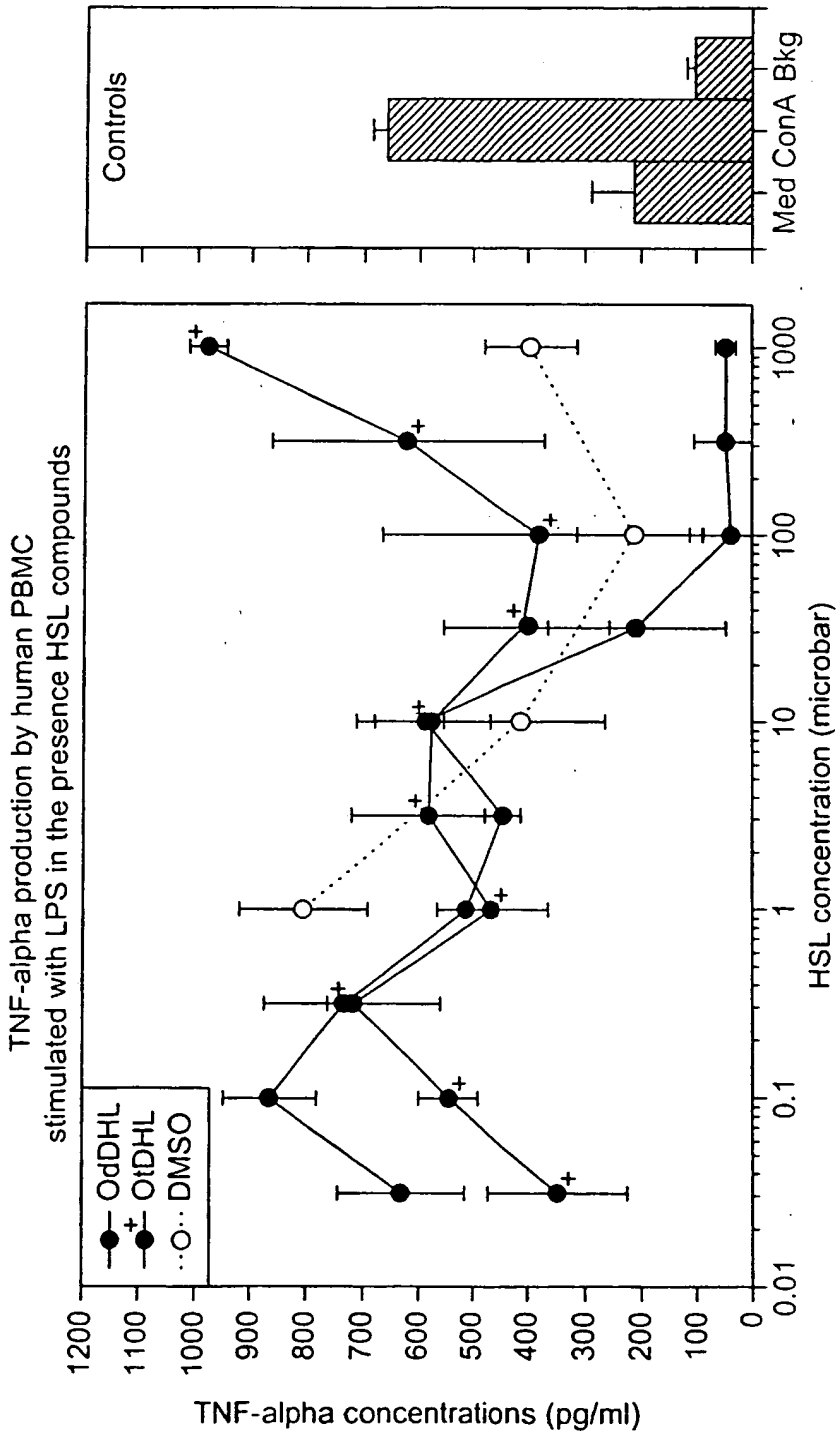

Controls
Med ConA Bkg
TNF-alpha production by human PBMC
stimulated with LPS in the presence HSL compounds
OdDHL
OtDHL
DMSO
TNF-alpha concentrations (pg/ml)
HSL concentration (microbar)
0.01
0.1
1
10
100
1000
0
100
200
300
400
500
600
700
800
900
1000
1100
1200

FIG. 10

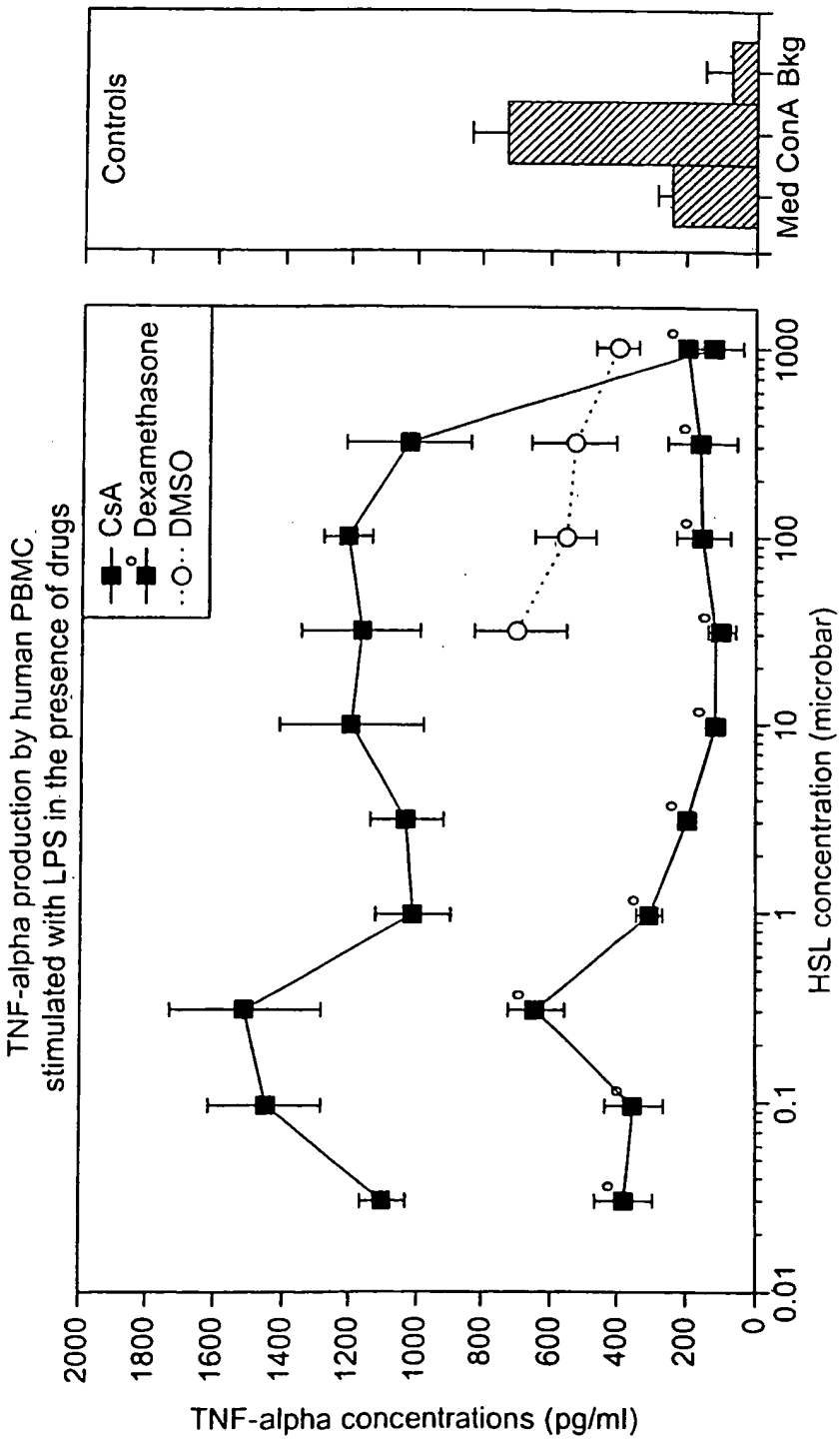

Controls
Med ConA Bkg
TNF-alpha production by human PBMC
stimulated with LPS in the presence of drugs
CsA
Dexamethasone
DMSO
TNF-alpha concentrations (pg/ml)
HSL concentration (microbar)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0.01
0.1
1
10
100
1000

FIG. 11

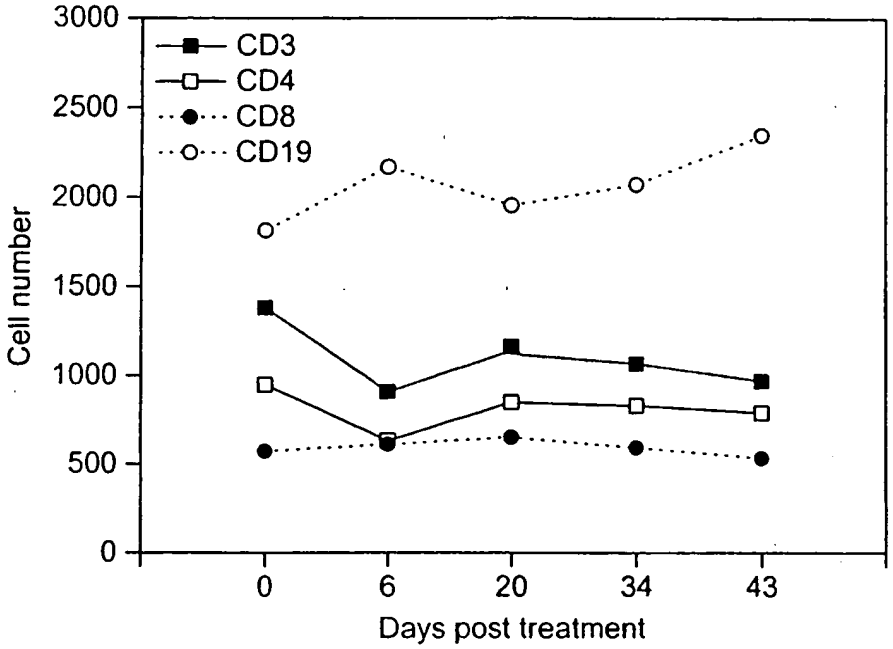
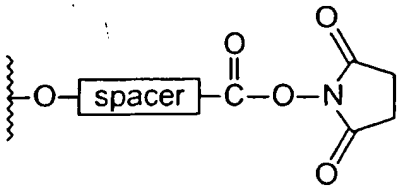


FIG. 12

Construction of the Affinity Matrix for the
Isolation of Binding Proteins (Immunophilins)



Affi-Gel 10 or 15 (activated affinity support)

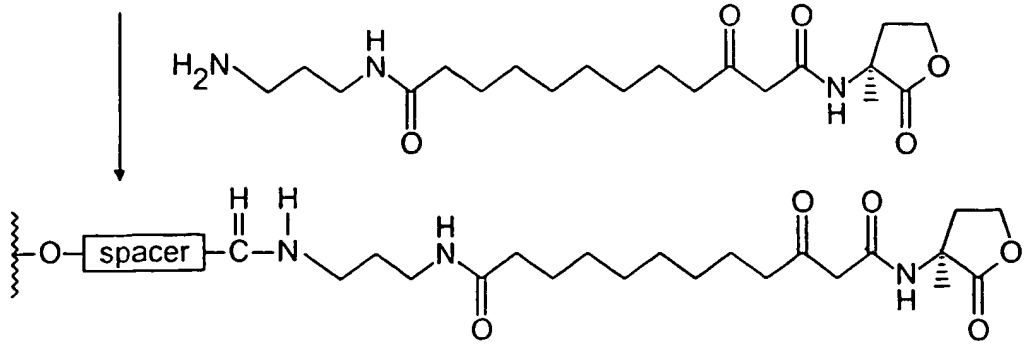
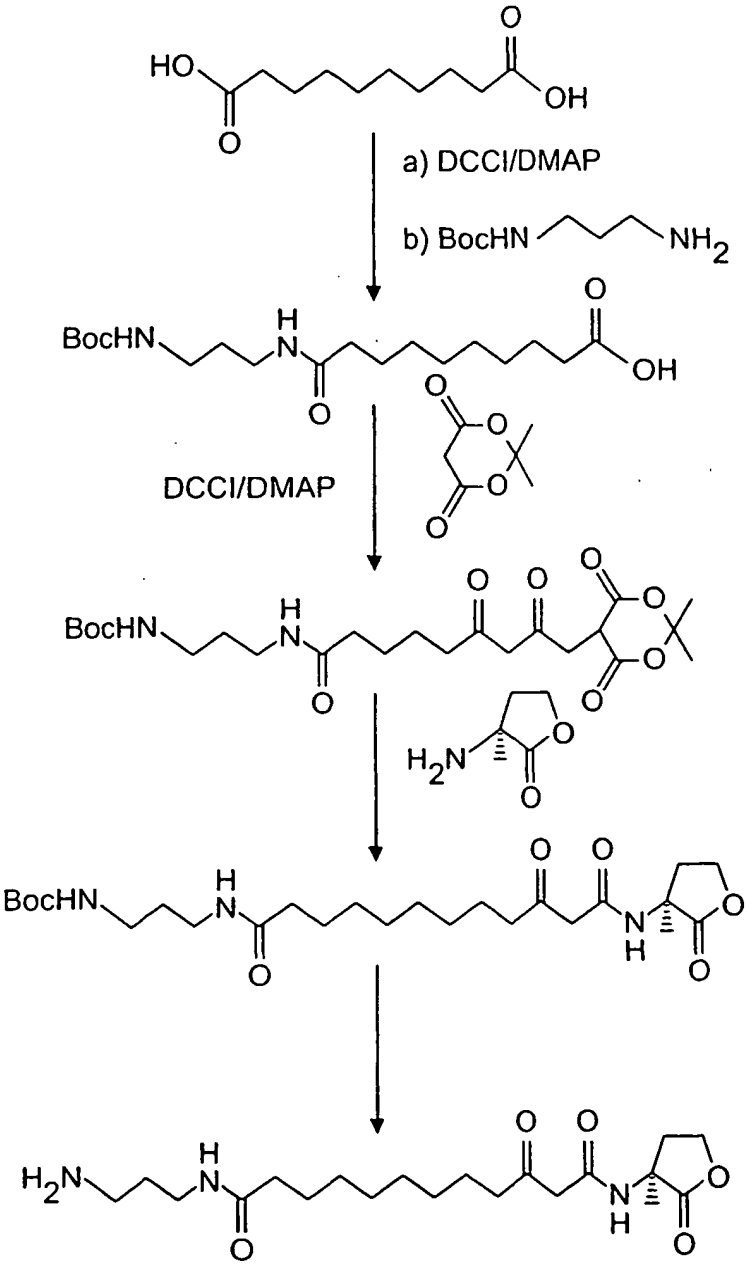


FIG. 14

Synthesis of terminally functionalised OdDHL (3-oxo-C12-HSL) - Ligand for Affinity Chromatography



Abbreviations
dicylohexylcarbodiamide
4-dimethylaminopyridine
butoxycarbonyl
trifluoroacetic acid

FIG. 13

INTERNATIONAL SEARCH REPORT

Internal ɔlication No
PCT/GB 02/04191

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D307/33 A61K31/365 A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 74801 A (UNIVERSITY OF NOTTINGHAM) 11 October 2001 (2001-10-11) cited in the application page 24 -page 27; claims 1-13	1-23
X	WO 01 26650 A (THE UNIVERSITY OF NOTTINGHAM) 19 April 2001 (2001-04-19) page 1 -page 7; examples 1-4	1-13,19, 23
X	WO 99 27786 A (THE UNIVERSITY OF NOTTINGHAM) 10 June 1999 (1999-06-10) page 5, line 4 -page 9	1-13,19, 23
X	WO 98 57618 A (THE RESEARCH AND DEVELOPMENT INSTITUTE, INC.) 23 December 1998 (1998-12-23) page 6, line 30 -page 10, line 3 page 13 -page 17	1-13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2003	04/02/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kyriakakou, G

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 01175 A (THE UNIVERSITY OF NOTTINGAM) 12 January 1995 (1995-01-12) cited in the application page 18 -page 19; claims 1-18	1-23
A	WO 92 18614 A (THE UNUVERSITY OF NOTTINGHAM) 29 October 1992 (1992-10-29) cited in the application the whole document	1-23
A	US 5 591 872 A (JAMES P. PEARSON ET AL.) 7 January 1997 (1997-01-07) cited in the application the whole document	1-23
X	NEIL D. ROBSON ET AL.: "Bacterial N-Acyl-homoserine-lactone-dependent signalling and its potential biotechnological applications" TRENDS IN BIOTECHNOLOGY., vol. 15, 1 November 1997 (1997-11-01), pages 458-464, XP004092668 ELSEVIER PUBLICATIONS, CAMBRIDGE., GB ISSN: 0167-7799 page 460; table 1	1-13
Y	GIANPAOLO PAPACCIO: "Prevention of low dose streptozotocin-induced diabetes by acetyl-homocysteine-thiolactone" DIABETES RESEARCH AND CLINICAL PRACTICE, vol. 13, 1991, pages 95-102, XP001005389 AMSTERDAM, NL ISSN: 0168-8227 cited in the application the whole document	1-23
Y	GARY TELFORD ET AL.: "The Pseudomonas aeruginosa Quorum-Sensing Signal Molecule N-(3-Oxododecanoyl)-L-Homoserine Lactone has Immunomodulatory Activity" INFECTION AND IMMUNITY., vol. 66, no. 1, January 1998 (1998-01), pages 36-42, XP001007928 AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON., US ISSN: 0019-9567 the whole document	1-23

INTERNATIONAL SEARCH REPORT

Inte al application No.
PCT/GB 02/04191

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Internal application No
PCT/GB 02/04191

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			EP	1268459 A1	02-01-2003
			WO	0174801 A1	11-10-2001
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			EP	1220671 A2	10-07-2002
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			EP	0994961 A2	26-04-2000
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			EP	0580692 A1	02-02-1994
			WO	9218614 A1	29-10-1992
			JP	6506588 T	28-07-1994
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US 5591872	A	07-01-1997	US	6057288 A	02-05-2000